

Sporogenesis and Sex Determination in *Begonia*
Schmidtiana

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A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN THE UNIVERSITY OF MICHIGAN

Reprinted from the AMERICAN JOURNAL OF BOTANY, Vol. XIX, No. 4, pp. 365-384,
April, 1932



SPOROGENESIS AND SEX DETERMINATION IN *BEGONIA SCHMIDTIANA*¹

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(Received for publication June 23, 1931)

The following study of *Begonia Schmidtiana* Regel was originally undertaken as an investigation in sporogenesis, but because of the remarkable findings it later developed into an investigation of the problem of the determination of sex in a monoecious plant. The plant was selected from among a number of species of *Begonia* in cultivation at the Botanical Gardens of the University of Michigan chiefly because of the abundance of its flowers which made possible the collections of large amounts of cytological material from a single plant. The results lead to the exclusive study of this species, but it is hoped that investigations may be continued in other species of the genus and among related forms.

MATERIAL AND METHODS

The material was collected from the Botanical Garden chiefly during the summer months since at this season flowers are more abundant than at other seasons of the year.

Several fixing fluids were employed for different purposes. Strong Flemming's solution diluted with an equal part of water gave satisfactory preparations for some stages but it drew the chromosomes so closely together that accurate counts could not easily be made. A formol-acetic-alcohol mixture (commercial formalin, 2.6 cc.; glacial acetic acid, 1 cc.; 50 per cent alcohol, 40 cc.) gave preparations having good chromosome spacing, but with serious shrinkage of the cell contents. For chromosome counts and general purposes, the fluid that gave the best results was the following modification of Bouin's solution;

Bouin's solution.....	100.00 cc.
Chromic acid.....	.50 g.
Urea.....	1.00 g.

Material was left from 8 to 10 hours in the fluid, then rinsed with water and carried through a long series of alcohols. The stain most generally employed was iron-alum haematoxylin.

The following measurements will give an idea of the size of the nucleus and the nucleolus, and of the minute chromosomes of *Begonia*:

	Nucleus	Nucleolus
In the resting cells of the archesporium.....	3.95-5.52 μ	1.58-2.32 μ
In prophase of heterotypic mitosis.....	5.52-7.89 μ	1.58-2.32 μ
In synizesis.....	6.32-9.47 μ	2.37-3.16 μ

¹ Papers from the Department of Botany, University of Michigan, No. 359.

Measurements of the chromosomes shortly before metaphase of the heterotypic mitosis;

1st type = 2.37 μ long, and .592 μ wide
 2nd " 1.975 " " .685 " "
 3rd " 1.58 " " .79 " "
 Odd chrom. 4.25 " " .708 " "

Chromosomes are most easily measured shortly before they become arranged to form the equatorial plate on the heterotypic spindle. At metaphase of this mitosis the bivalents lie so closely together that it is difficult to distinguish them. At anaphase the chromosomes are more condensed than at metaphase.

Chromosome counts were made from the various parts of the plant substantially as recorded in the accompanying table.

TABLE 1. *Number of Chromosome Counts*

Chromosome Counts	♂ Flower 12 Chrom.	♀ Flower 13 Chrom.	Vegetative Sporo- phyte 13 Chrom.	Gametophyte		Endo- sperm 13 Chrom.
				♂ 6 Chrom.	♀ 6 or 7 Chrom.	
Growing point.....	50	50	50			
Developing petal.....	5	10				
Pedicel.....	10	10				
Developing stamen.....	50					
Epidermis of anther.....	10					
Tapetum.....	3					
Bract.....		10				
Developing ovule.....		100				
Nucellus.....		5				
Embryo.....			20			
Young leaves.....			30			
Root tip.....			20			
Hair.....			3			
Metaphase of heterotypic mitosis.....	300	200				
Anaphase of heterotypic mitosis.....	200	75				
Interkinesis.....	75	50				
Homoeotypic metaphase.....	150	100				
Microspore.....				100		
Megaspore.....					50	
Developing egg.....					15	
Endosperm.....						30

THE CHROMOSOMES OF THE STAMINATE AND PISTILLATE FLOWERS

Begonia Schmidiana has chromosomes of 4 types when classified according to size and all are more or less curved like the letter "C." Four chromosomes (two pairs) are wider and longer than the others, four chromosomes (two pairs) are medium in size, and another four (two pairs) somewhat shorter than those of medium length and slightly broader. These three types are present in all parts of the plant. The fourth type is unpaired and

because of its absence in the staminate flower, it seems probable that this chromosome has a bearing on the determination of sex. It is coiled in form, tapering to a point, and resembles a question mark (Pl. XXXI, figs. 60 s, 61 s).

The sporophyte of the plant therefore carries 13 chromosomes in all tissues excepting those of the staminate flower where the number is 12. Counts of 13 chromosomes have been made in the root tip (Pl. XXVI, fig. 1), the embryo (fig. 2), the hair of the stem (fig. 3), the tip of the stem that will become the pistillate flower (fig. 4), the tissues of the ovule before the megaspore is formed (fig. 5), the petal and all the other parts of the pistillate flower, and the bud of the stem.

The staminate flower carries 12 chromosomes in all of its parts as shown by the counts from the flower bud initial (fig. 6), petal (fig. 7), the epidermis of the anther (fig. 8), archesporium initial (fig. 9), pedicel, bracts, and other regions.

The staminate and pistillate flowers arise close together from a common axis (text fig. 1). By horizontal cell divisions this axis divides dichotomously to give rise to the staminate and pistillate flower buds (text fig. 2).



TEXT FIG. 1. The origin from a common axis of the staminate flower (S) and the pistillate flower (P).

TEXT FIG. 2. Older stages in the development of the staminate flower (S) and the pistillate flower (P), showing dichotomous arrangement.

The dichotomous arrangement is, however, not very evident due to the fact that the staminate flower grows and matures faster than the pistillate and often drops from the plant three or four days before the latter opens. At the point of separation of the staminate and the pistillate flower buds the

unpaired chromosome apparently does not divide as do the others but goes as a whole into the bud that is to become the pistillate flower. Hence the staminate flower bud is deprived of that chromosome.

Němec (1904), followed by Häcker (1904), Woycicki (1906), and Schiller (1909), first reported the occurrence of heterotypic divisions in somatic cells as the result of treatment with chloral hydrate and other chemicals, but so far as the writer is aware this type of somatic reduction of chromosomes has not heretofore been reported as normal behavior, and the conclusions of this paper have been reached only after examining numerous specimens and finding no exceptions to the above statement of chromosome counts. However, the possibility that such a reduction takes place has been suggested.

Bond (1915) studied the sex characters of abnormal *Begonia* flowers, and found among other things that the "instability of equilibrium in the primary sex elements" is associated with the instability in somatic tissues. Thus an abnormal floral bract occurs on the pedicel of an abnormal flower and marks the place where the separation of the staminate and pistillate flowers would have occurred normally. Evidence was offered to prove that segregation of the sexes occurred in the meristematic part of the flower stalk and that "sex differentiation is a matter of qualitative cell division in somatic as well as in germinal cells." He stated further that sex dimorphism depends upon the kinds of units involved and their period of occurrence. Thus, in dioecious plants the differentiating cell division occurs in the germ cell, in monoecious plants at a later stage during flower formation, and in hermaphrodite plants at a still later stage during the development of sex organs. Finally he suggested that "if future cytological research should associate the male (or the female) sex in the *Begonias* with the presence of an accessory chromosome, then the position taken up by that chromosome in the qualitative cell divisions which divide the growing cell into end and lateral daughter cells will determine the primary sex of the flower just as the passage of such an accessory chromosome into one of the two alternative germ cells determine the sex of the individual."

Shaffner (1919) found in the dioecious plant *Cannabis sativa* L. that when it is grown out of season, with deficient light on a shallow soil, the female heredity which was at first active became suppressed giving away to the male inheritance which then became active. He concluded that "the change takes place in the vegetative body and is plainly caused by an internal change of the physiological state or condition of the meristematic tissues from which the flowers are produced." This statement clearly signifies a segregation of the sexes at some point in the meristematic tissue of a dioecious plant that becomes monoecious due to the action of environment although there is considerable time between the expression of the two sexes.

In view of these conclusions the present findings in *Begonia* are not without the support of other investigators.

MICROSPOROGENESIS

The archesporium arises from the third layer of cells below the epidermis (fig. 10). This line of cells divides to form a layer on the outside and the sporogenous tissue within. The layer, now third under the epidermis (Pl. XXVII, fig. 11), divides lengthwise to form a fourth layer and this becomes the tapetum surrounding the sporogenous tissue. In some cases a second division takes place, making the tapetum the fifth layer from the epidermis.

As the sporogenous tissue develops, the epidermal layer of the anther, and to a lesser extent the one just below it, becomes impregnated with a yellowish substance. The third layer loses its cell contents, and the tapetum stains deeply. In mature anthers only the tapetum and the second layer together with the epidermis remain, because the other cells between are absorbed apparently with the growth of the tapetum.

The sporocytes in a locule are usually in the same stage of meiosis, which however is generally a different stage from those of other locules in the same anther. There is therefore a wide range of stages in a single male flower which contains about 28 small stamens. Usually the outer stamens of the group mature earlier than the inner ones.

Prophase of Meiosis

A mature microsporocyte ready for meiosis contains a large nucleus occupying about one half of the cell cavity (fig. 12). The nucleolus lies at one side of the nucleus, and in fixed material it is surrounded by a clear area (fig. 13). The nucleus likewise lies at one side of its cell. The asymmetrical position of the nucleus persists through all stages, even up to the pollen tetrads. The nucleus contains a fine chromatic reticulum (fig. 14).

Certain strands of this chromatic network thicken coincident with the absorption of others and deeply staining threads become established (fig. 15). At this time and in later stages it is clear that the thread system is attached to the nucleolus (figs. 14, 16). No pairing of threads has been observed at this period of meiosis.

Synizesis

The thickening and condensation of the threads continues and the thread system gradually draws away from the nuclear membrane (fig. 16). The nucleolus frequently elongates at the point where it is attached to the thread system, thus becoming somewhat pear-shaped. Material appears to pass from the nucleolus into the threads (fig. 17), and the nucleolus stains much less deeply indicating that it has lost much of its contents. Masses of chromatin appear around the nucleus in grape-like clusters (fig. 18). The rest of the thread system which has not thus condensed gathers towards the contracted mass (Pl. XXVIII, fig. 19), and the nucleus passes into the condition of mid-synizesis (fig. 20) with all of the chromatic material contracted around the nucleolus and generally at one side of the nucleus. This condition persists for a long time, perhaps longer than any other stages of meiosis.

Frequently, before synizesis, the nucleolus may appear like a top around which a thread has been wound; or, it may resemble a fan-shaped arrangement of the threads. These conditions support the view of a definite connection between the thread and the nucleolus as reported by Van Camp (1924) for *Clivia miniata*, and Cleland (1924) for *Oenothera franciscana sulfurea*.

The nucleus emerges from synizesis through the gradual expansion of the thread system, as is illustrated by the figures 21 and 22. Finally an apparently continuous thread becomes evenly distributed through the nucleus, and the nucleolus, now staining more deeply, passes to the center (fig. 23). This is the stage of the open spireme.

Synapsis and Segmentation of the Spireme

The spireme gradually thickens in certain regions between which the thread becomes thin as though material had passed into these denser regions (fig. 24). Some of the threads come to lie parallel, in certain cases apparently because the sides of the loops approach one another (fig. 25), and frequently these threads lie so closely together as to twist about one another (figs. 26, 27). This seems to be the period of synapsis, since at this time homologous chromosomes become associated in the preparation for the later bivalents.

The drawing out of the thinner portions of the thread results in a segmentation of the spireme with the thicker regions as centers of chromosome organization. It is difficult to count the segments because of their varied forms and because the process of condensation generally brings them into confused groups, but the number seems to correspond closely to the chromosome count of 6 bivalents. This is the stage of second contraction.

Diakinesis

This stage really begins during the period of second contraction when the paired threads may be recognized in a state of synapsis. The paired threads may still be observed while the chromosomes are in an early stage of condensation (figs. 26, 27). Later it becomes evident that each thread gives rise to a chromosome (fig. 28). This is the true stage of diakinesis.

Although the chromosomes gradually become shorter and thicker they still retain a spiral form. Remnants of the linin threads may be observed attached to chromosomes (fig. 29). The chromosomes now become evenly distributed throughout the nuclear cavity and as they continue to shorten the spiral twists disappear. Finally the chromosomes assume their normal curved form and the association in pairs becomes less evident (Pl. XXIX, fig. 30).

Spindle Formation

After the condensation of the chromosomes the nuclear membrane gradually breaks down. The nuclear space becomes oval in form and spindle fibers begin to appear (fig. 31), becoming conspicuous even before the meta-

phase stage. For this reason when the chromosomes are grouped on the spindle in preparation for metaphase (fig. 32), they may give the impression of irregular arrangement. This irregularity is, however, shortly followed by a grouping of the chromosomes in pairs (figs. 33, 34) preliminary to the metaphase figure.

Metaphase and Anaphase

After the spindle is formed the homologous chromosomes are found closely paired, and arranged on the equatorial plate. The association is so intimate as sometimes to conceal the identity of each homologue (figs. 35, 36), and the individual chromosomes of each bivalent are difficult to recognize. They are most clearly shown in polar views of the equatorial plate (fig. 37) in metaphase.

The arrangement of the chromosomes follows the usual pattern for the heterotypic mitosis and anaphase presents no exceptional features. One half of the chromosome complement passes to each pole of the spindle (fig. 38) as reduction takes place. Metaphase and anaphase may frequently be found in the same locule of the anther.

Telophase and Interkinesis

During anaphase of the heterotypic mitosis there appears the first evidence of a lengthwise fission of each chromosome in preparation for the homoeotypic division (fig. 39). Therefore six split chromosomes are carried into telophase. The halves of the chromosomes at first remain attached at their ends forming V's but later become quite separated from one another (fig. 40) except for thread connections that form an imperfect network within the nucleus (figs. 41, 42).

Interkinesis is a well-defined period between the heterotypic and the homoeotypic mitoses during which the nucleus again acquires a nucleolus. The halves of the split chromosomes lie so far apart that 12 chromosomes are easily counted. It is difficult to recognize that association of the halves of split chromosomes which is so commonly found at this period of meiosis in many plants.

Homoeotypic Mitosis

The homoeotypic mitosis presents no important distinctive features. The halves of split chromosomes become associated during prophase (Pl. XXX, fig. 43) and at metaphase come so closely together that they are not easily recognized (fig. 44), and then are distributed, 6 chromosomes passing to each pole (fig. 45). During telophase the chromosomes elongate (fig. 46) and presently form a close network of delicate threads.

Cytokinesis

During synizesis the cytoplasm starts to withdraw from the cell wall. Contraction begins at the corners of the cell (Pl. XXVIII, fig. 19), thus rounding off the edges of the protoplast, which then becomes much smaller in

volume in comparison with the size of the nucleus. By a later breaking down of the primary walls the cells of the sporogenous tissue become separated and finally lie freely in the fluid of the pollen sac. A secondary wall gradually forms and thickens as sporogenesis proceeds until it becomes a conspicuous structure in later stages of meiosis (fig. 47). Castetter (1925) found such a wall in *Melilotus* which he thought to be a callose secretion of the sporocyte.

Cytokinesis results from a cleavage of the cytoplasm by furrowing, similar to that described by Farr (1916) in *Nicotiana*. In the telophase of the heterotypic mitosis, thickenings clearly visible under the low power objective were observed at the median plane of the spindle (fig. 47). These however are not concerned in the formation of a wall. Following the homoeotypic mitosis fibers were found connecting the four daughter nuclei (fig. 46). Vacuolization occurs in the equatorial plane of these fibers (fig. 48) as has been observed by Castetter (1925) in *Melilotus*. Furrows from the periphery cut inward through these vacuoles and, meeting at the center of the cell, divide the protoplast into four tetrahedral daughter cells (fig. 49). Extensions of the secondary wall follow the planes of the cleavage furrows. The secondary wall is of temporary nature since it disorganizes before the pollen grains reach maturity (fig. 50).

MEGASPOROGENESIS

Development of the Ovule and of the Integuments

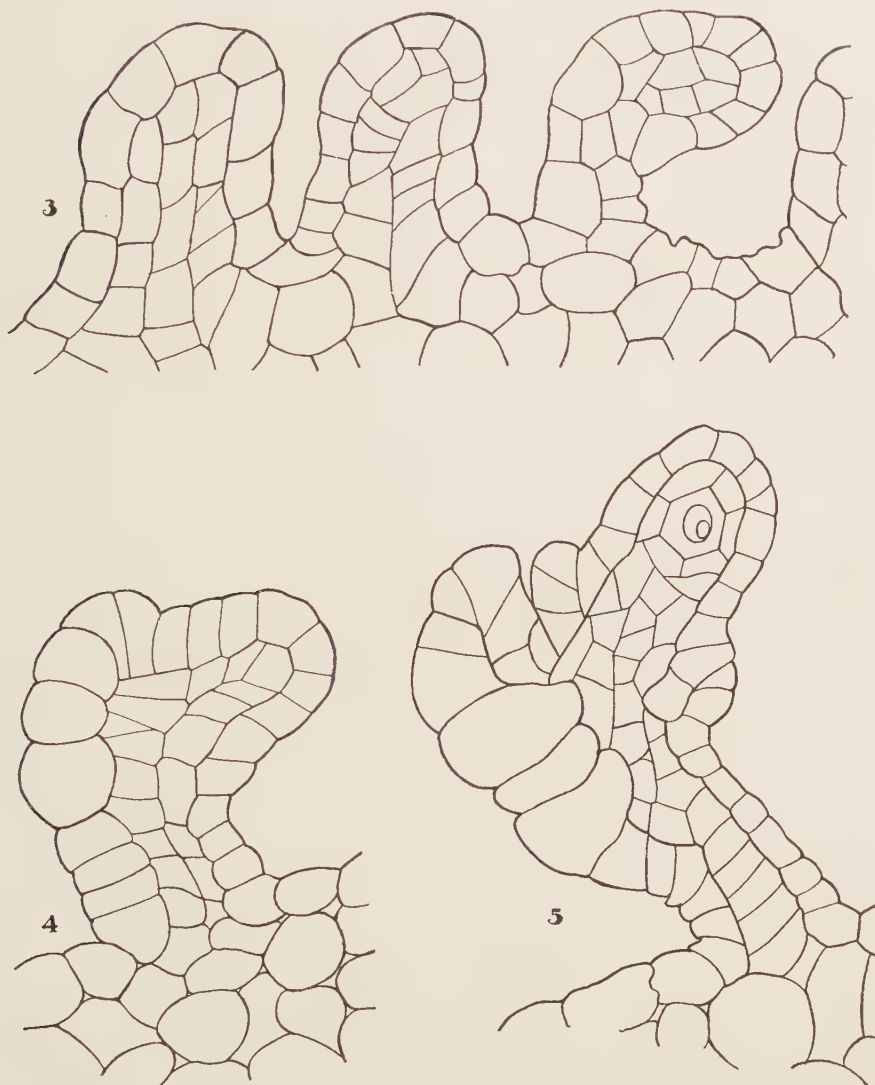
During the formation of the ovules the placenta stains deeply because the cells become filled with globules of food material. Swellings which later become ovules arise subepidermally from the placenta. A young ovule is at first an outgrowth composed of a few rows of cells (text fig. 3). Rapid cell divisions at its tip differentiate an enlarged upper portion from a thin stalk. Further divisions considerably enlarge the tip which then begins to curve towards the placenta (text fig. 4). As the ovule bends, the epidermal cells in its dorsal region increase in size and the inner and outer integuments arise from this dorsal portion of the ovule (text fig. 5).

The outer integument grows more rapidly and its outer layer is composed of very large cells. The inner integument made up of small cells develops more slowly but finally grows beyond the tip of the nucellus leaving only a minute micropyle. The final result of the development is an anatropous ovule (text fig. 6).

Meiosis

The megasporocyte is differentiated in the nucellus as the second cell below the epidermis and is easily recognized after the integuments begin to appear (text fig. 5). It becomes a large cell as the ovule develops. The history of meiosis is the same as in the microsporocyte except that in the megasporocyte the odd chromosome is present. This chromosome can be

recognized at the metaphase of the heterotypic mitosis as an unpaired coiled chromosome (Pl. XXXI, figs. 60 s, 61 s).



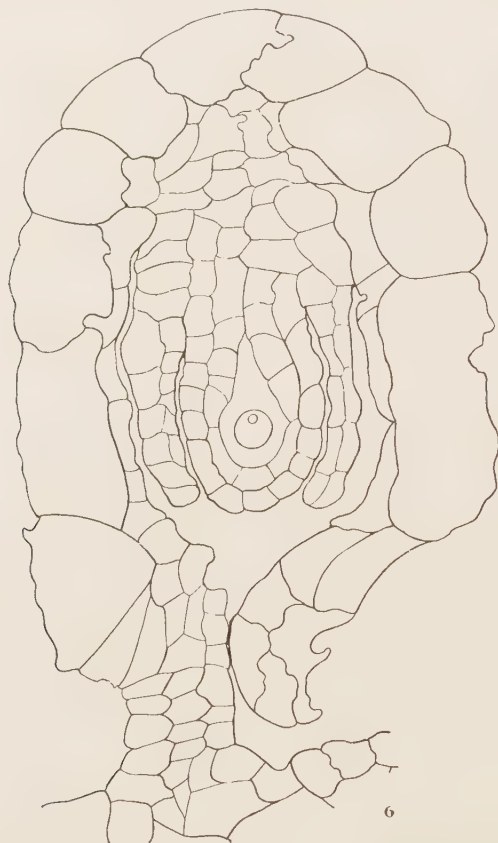
TEXT FIG. 3. Early stages in the development of the ovule.

TEXT FIG. 4. Older ovule beginning to curve, integument starting to develop.

TEXT FIG. 5. Ovule with definitely formed megasporocyte and integument initials.

The early stages of meiosis take place during the development of the ovule and the heterotypic metaphase does not occur until the ovule is at least as old as that shown in text figure 6. The prophase of meiosis follows the same history as in the microsporocyte. There is a long period when the nucleus is in synizesis (Pl. XXXI, figs. 51, 52) followed by the formation of a

spireme on which thickenings may be observed where the chromatin material seems to collect (fig. 53). Loops are developed and the threads pair with one another (fig. 54). Segmentation of the spireme follows as a result of the drawing out of the thinner portions of the threads. There is then a second contraction of chromatic material (fig. 55) during which synapsis is continued by a further twisting of the paired threads (fig. 56). Soon the pairs



TEXT FIG. 6. Ovule with megasporocyte in early stage of meiosis, outer integument covering the inner integument.

loosen, each thread becoming a chromosome which at first retains the spiral twists of synapsis (fig. 57).

The chromosomes then shorten and assume their usual form (fig. 58). During the formation of the spindle the homologous chromosomes become arranged in pairs (fig. 59). Metaphase follows and in this stage the bivalent chromosomes are easily counted and their form and size determined, the odd chromosome *s* being easily recognized by its spiral form (figs. 60, 61). A lengthwise splitting of the chromosomes in preparation for the homoeotypic mitosis gives V-shaped structures during telophase that persist into

interkinesis (fig. 62). Soon the homoeotypic division takes place (Pl. XXXII, figs. 63, 64) and the four megaspores are formed.

Our chief interest in this history concerns the behavior of the odd chromosome which makes the count in the megasporocyte 13 in contrast with the count of 12 in the microsporocyte.

During anaphase of the heterotypic mitosis when the homologous chromosomes have segregated, the odd chromosome is found to pass to the pole nearest the micropyle. Because of the splitting of the autosomes the two nuclei following the heterotypic mitosis contain 13 and 12 chromosomes, respectively (fig. 62). These two nuclei usually divide simultaneously in the homoeotypic mitosis (fig. 63), although the mitosis nearest the micropyle may proceed more rapidly (fig. 64). The four megaspores which result are arranged in a line (fig. 65), the two inner nuclei of the series having six and the two nearest the micropyle having seven chromosomes. The egg nucleus of the embryo sac carries the odd chromosome.

DISCUSSION

As in other characters of an organism, sex determination depends on two principal factors, heredity and environment. There seems little doubt that both factors take part in the determination of sex, in spite of some extreme views which recognize only one of the factors. Many investigations have been made on the role played by these factors, with remarkable results. A few examples will be cited and the influence of environment on sex will be considered first.

As early as 1879 Prantl observed that strong light caused the production of female prothallia in ferns, while weak light caused the production of male prothallia. Later in 1881 he showed that strong light and abundant food supply induced the production of archegonia and the reverse condition the production of antheridia in *Equisetum*. Buchtien (1887) supported these conclusions, but Darling (1909) pointed out that the prothallia with which Prantl worked were not strictly dioecious but had only a tendency to dioeciousness. The important studies of Klebs (1896) proved that environment determined the production of vegetative branches, zoöspores, or gametes in certain algae. Cutting (1910) showed that sex in *Marchantia*, apparently so stable, is not wholly determined by segregation. That sex in ferns is influenced by environment has been shown by the experiments of Wuist (1913) on *Onoclea*, Nagai (1915) on *Osmunda* and *Asplenium*, and Czaja (1921) on several other species.

Shaffner (1919-1928) has made extensive reports on the influence of environment on sex, studying the effects of age, nutrition, light, etc., in *Cannabis sativa*, *Morus alba*, *Salix amygdaloides*, *Humulus japonica*, *Thalictrum dasycarpum*, and *Arisaema Dracontium*. His conclusions were positively in favor of the environmental factors as modifiers of sex. He was able to reverse the sex of the dioecious plant hemp, and to produce intersexes

or only one sex by changing environmental conditions of the plant. However, he does not disregard the internal mechanism as a factor collaborating with environment in the determination of sex.

It is claimed by Ciesielski (1911) that the age of pollen has an influence on the sex of hemp, fresh pollen grains giving rise to seeds producing male plants, and old pollen grains to female plants. However, Bessey (1928) working with hemp found that the age of pollen had no effect on the sex of the resulting offspring.

That environment affects the chromosomes has been demonstrated by Belling (1924-1925) who showed that warmth decreased the number and size of chromosomes in *Cypripedium acaule*, and that cooling at night and changes of temperature for three years caused chromosomal mutations in *Uvularia*. Hirata (1927) claimed that sex in hemp is due to *XY* chromosomes and may be influenced by environment through its action on enzymes affecting sex.

In animals, the effects of environment on sex have also been studied. Riddle (1912) found that the kind and amount of food modified the sex of pigeons, and that eggs of small size, high water content, and small energy content are male-producing while those of opposite characters are female-producing. Hertwig (1912) working in frogs stated that over-ripe eggs produced an excess of male offspring and vice versa. Lillie (1917) reported for twins in cattle that the secretion of a certain hormone by a male embryo arrests the normal development of an accompanying female, producing the sterile "free martin." Minoura (1921) found that grafting of reproductive tissues changes the sex of the chick, but that grafts from liver and other non-sexual organs had no effect.

On the other hand, proofs that sex is determined through inheritance are overwhelming. The idea was first suggested by Mendel himself. The first demonstrations were made through animals.

In 1891 Henking observed additional chromatin material which he thought to be a nucleolus in half of the spermatids of *Pyrrhocoris*. Other investigators, McClung (1899), Paulmier (1899), and Montgomery (1901), reporting similar conditions in other insects such as *Anasa*, *Protenor*, and species of grasshoppers, discovered this body to be an accessory chromosome and McClung associated it with the determination of sex.

It was later found that sex determiners may occur in pairs, and may be located in the male or in the female individual. When the male carries the sex chromosome it produces two kinds of sperms, as in the *Lygaeus XX-XY* type described by Montgomery (1901). The female may behave in the same way, as in the moth *Abraxas grossulariata ZW-ZZ* type described by Doncaster (1913-1914), or the *ZO-ZZ* type in *Taloepora tubulosa* described by Seiler (1917). Cytological and genetic evidence has firmly established the presence of sex chromosomes in many animals and an extensive literature had been developed about the subject, recently reviewed by Schrader (1928).

Sex chromosomes have been located, and described as to their behavior, size, and shape, and environmental factors affecting them have been determined.

In plants the idea of a Mendelian inheritance of sex was early supported by Strasburger (1900), Bateson and Saunders (1902), and Castle (1903). In 1906, Blakeslee observed that sexual segregation occurred in *Phycomyces nitens*. This was shown by Burgeff (1914-1915) to be due to nuclear differences in the hyphae. The Marchals (1907) demonstrated that in certain mosses internal mechanism rather than environment determined sex and that segregation of the sexes occurred at meiosis. Correns (1907) found two kinds of pollen grains in the dioecious plant *Bryonia*, but only one kind of egg. Strasburger (1909) observed that of the four spores of a tetrad in *Sphaerocarpos* two developed male and two female gametophytes. It was suggested that the development of distinct male and female plants from the tetraspores of *Polysiphonia* indicates a segregation of sex factors in sporogenesis. These illustrations indicate that segregation of the sexes takes place in the life cycle of some plants through nuclear mechanism. Sex chromosomes in plants were, however, not identified until Allen (1917) discovered them in *Sphaerocarpos Donnellii*.

In certain dioecious angiosperms several investigators through genetical studies reported two types of pollen grains. Strasburger (1910) suggested the possibility of such condition. Correns (1907) in *Bryonia* and Shull (1910) in *Lychnis* demonstrated that male plants were heterozygous for sex.

In 1923 investigators working on four different dioecious plants demonstrated two types of pollen grains differing in the presence or absence of an accessory or sex chromosome, thus presenting the same situation as is found in certain animals. This was shown by Santos for *Elodea*, Kihara and Ono for *Rumex*, Winge for *Humulus*, and Blackburn for *Melandrium*. Later studies by Meurman (1925) have added several species of *Populus* to the list. These cytological studies on plant material give support to the suggestions of earlier workers, Strasburger (1900), Correns (1907), and Shull (1910), that sex is determined through a mechanism of inheritance.

In conclusion, the following quotations may serve to express the prevailing opinions on the determination of sex. Thomson (1913) states that "if nutritive and other environmental influences are operative, it is, in the main, by affecting the production and survival of sexually predestined germ-cells." Sharp (1926) expresses the opinion that "under ordinary conditions it is the genetic factors that are differential," and that "the effects of heredity and environment are not so much opposed as complementary." Wilson (1928) affirms that "investigations upon the nature of sex were long dominated by the preconceived notion that it is determined by conditions external to the germ, but the inadequacy of this view is now conclusively demonstrated. . . . It seems clear, however, that external conditions operate merely to incite the development of one set of sexual characters at the cost of the opposite set in an organism that is actually or potentially monoe-

cious or hermaphroditic." In dioecious organisms, "both the genetic and cytological evidence demonstrates the existence in the germ-cells of a definite internal mechanism that is adjusted for an automatic production of the sexes under identical external conditions. It is probable, therefore, that in so far as external conditions affect sex production it is in all cases through their influence upon the internal mechanism."

It is not the purpose of this paper to claim that sex inheritance is purely Mendelian in character, for although more generally heredity appears to determine sex, the results have been modified by experimental control of environmental conditions. The two factors go hand in hand and sex is determined by their interrelation. The internal factors depend on the structure of chromosomes subject to the influence of hormones and enzymes, and these in turn are influenced by the environment under which the plant thrives. The external stimuli may either be favorable or lethal and the latter may vary in toxic effects from a mild inhibition to actual destruction. There is an interdependence of the individual organs together with a relation to an outside world. Chromosomes are vehicles of sex determination, but are subject to outside influences. Confirmation of the latter conclusion does not disprove the former statement.

It is here proposed that the accessory chromosome present in the pistillate and absent from the staminate flowers of the monoecious plant *Begonia* is a sex determiner of a like nature to that found in the pollen or spores of several dioecious plants, although its critical behavior as a determiner of the sex in *Begonia* occurs with the differentiation of the flower initials rather than at meiosis. Since it has been shown that sex chromosomes or determiners are present in both animals and plants, it is but natural to expect that they should be present in such a highly evolved plant as *Begonia*.

When sex determiners are carried by the male gamete, a permanent separation of the sexes is assured as in the case of animals and dioecious plants. Sex is acquired by chance because two kinds of male gametes give two different zygotes in the fertilization of the sexually similar eggs.

However, since the sex chromosome present in the monoecious plant *Begonia* can not be found in the male gamete, for in that case a dioecious condition would result, it must therefore be present in the female gamete so that the offspring will be constant to the monoecious character of the species. Fertilization then can give only one type of plant because there is only one kind of pollen grain to fertilize one kind of egg, that which bears the sex determiner.

Segregation of the sexes must take place before the formation of the flower, for should it occur later during the formation of sex organs, the flower would be hermaphroditic as pointed out by Bond (1915). The monoecious condition in *Begonia* is due to the fact that the segregation of a sex chromosome occurs before the formation of the flowers and that the presence of the sex chromosome determines the development of a pistillate flower and

its absence the development of a staminate. Therefore the present findings in *Begonia* are what might be expected with respect to the segregation of the sexes and the final location of the sex chromosome in the egg.

The gap between sex segregation in dioecious plants, which occurs in the germ cells, and that in the monoecious plant, which takes place during floral development, seems a wide one. In the evolutionary stages this has probably been bridged by the occurrence of sex segregation at different points of location intermediate between the two. One might venture to speculate that this is one reason why sex in monoecious plants may be unstable; for example Bond (1915) showed the presence of abnormal flowers with characters of both sexes.

A detailed study of chromosome counts in the family Begoniaceae would be interesting. Heitz (1927) made such a study in 15 of the genera. He counted 29–32 chromosomes in "*Begoniastrum Schmidiana*." This must be a different species from the *Begonia Schmidiana* of this paper. Heitz's counts indicate that his forms were polyploids, mostly tetraploids, and that the *Begonia Schmidiana* under consideration here is a more primitive type. Moreover, it is interesting to note that his counts for species were not constant, thus: he records 36–42 chromosomes in *Pritzelia scandens*, 28–30 chromosomes in *Augustias Dregei* and *Magnusia metalica* and 24–28 chromosomes in *Petermannia isoptera*, *Huszia Baumanii*, *Magnusia conchaefolia*, *Donaldia ulmifolia*, and unclassified varieties of *Java assamica* and *mexicana*. These numbers indicate that they are tetraploids whose chromosome numbers are multiples of 6 and 7, the counts in *Begonia Schmidiana* Regel. His other counts show that there is a great irregularity among the chromosome counts in different species. This is to be expected considering the extensive hybridization and cultivation that the plant has undergone.

Concerning sporogenesis and the life history in general, the following peculiarities deserve mention. Pairing of threads was observed after synzesis, in stages of the thicker spireme before diakinesis. Segmentation of the spireme is delayed until diakinesis. The connection of the nucleolus with the thread system is very evident.

The sex chromosome present in the sporophyte comes to enter the egg because it passes into the nucleus of the secondary megasporocyte that is nearest the micropyle. There seems to be a certain polarity of the heterotypic mitosis that directs this movement.

SUMMARY

1. The sporophyte has 13 chromosomes of four types. One is unpaired, and is absent from the staminate flower, which consequently has 12 chromosomes in contrast to the pistillate flower with 13 chromosomes.

2. The unpaired chromosome is probably concerned with the determination of sex. It fails to enter the stem initial from which the staminate flower is developed.

3. The prophases of meiosis show a definite connection between the chromatic thread system and the nucleolus from which material passes into the threads.

4. Parallel threads were not observed until after synizesis. The parallel threads become nodulated before segmentation, the regions between growing thinner until separation is effected, and the nodules become centers of chromosome organization.

5. The number of segments seems to correspond to the number of bivalents found in the microsporocyte, that is, 6 pairs. Later, in diakinesis, the segments are recognized as 6 pairs of twisted threads.

6. Diakinesis begins during the period of second contraction when the paired threads may be observed in a state of synapsis.

7. The association of the homologous chromosomes at metaphase of the heterotypic mitosis is sometimes so intimate as to conceal their identity in the bivalents.

8. The first evidence of a lengthwise division of each chromosome in preparation for the homoeotypic division appears during anaphase of the heterotypic mitosis.

9. During interkinesis the halves of the split chromosomes lie so far apart that the 12 chromosomes are easily counted.

10. The nucleolus, which disappears before the heterotypic mitosis, reappears rather suddenly during interkinesis as though it might be a reservoir of chromatin supply.

11. Cytokinesis is accomplished by cleavage furrows that cut inward from the periphery through vacuoles that lie between the four nuclei following the homoeotypic mitosis. Extensions of a secondary wall follow the planes of the cleavage furrows, but this wall disorganizes before the pollen grains reach maturity.

12. The megasporocyte is differentiated at the tip of the small nucellus. The details of meiosis in megasporogenesis are essentially the same as in microsporogenesis.

13. The odd chromosome is easily recognized at metaphase of the heterotypic mitosis by its spiral form. It passes to the pole nearest the micropyle with 6 of the 12 autosomes.

14. Because of the splitting of the autosomes the two nuclei of the interphase of megasporogenesis following the heterotypic mitosis have 13 and 12 chromosomes, respectively.

15. Following the homoeotypic mitosis the four megaspores are arranged in a line, the two nuclei nearest the micropyle having 7 chromosomes each, and the two inner nuclei 6 chromosomes. The egg nucleus of the embryo sac carries the odd chromosome, making a total of 7 chromosomes in its set.

The writer wishes to thank Professor H. H. Bartlett for suggesting the material, which was generously supplied by the Botanical Garden; and she

is greatly indebted to Dr. B. M. Davis, under whose direction the study was conducted, for important criticism, interest, and encouragement.

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EXPLANATION OF FIGURES

All drawings were made with the aid of a camera lucida under the Zeiss apochromatic oil immersion, pri. mag. 120, N.A. 1.3, with compensating ocular $\times 20$, draw tube at 171 mm. Figures reduced one-third in reproduction.

PLATE XXVI

- FIG. 1. Cell from root tip, 13 chromosomes.
- FIG. 2. Cell from the embryo, 13 chromosomes.
- FIG. 3. Cell from the hair of the stem, 13 chromosomes.
- FIG. 4. Cell from the pistillate flower initial, 13 chromosomes.
- FIG. 5. Cell from the nucellus of ovule, 13 chromosomes.
- FIG. 6. Cell from the staminate flower initial, 12 chromosomes.
- FIG. 7. Cell from the petal of staminate flower, 12 chromosomes.
- FIG. 8. Cell from epidermis of the anther, 12 chromosomes.
- FIG. 9. Cell from archesporial initial, 12 chromosomes.
- FIG. 10. Archesporium in anther showing its origin from the third layer of cells below the epidermis.

PLATE XXVII

- FIG. 11. Sporogenous tissue with surrounding tapetum.
- FIG. 12. Resting cell of the sporogenous tissue.
- FIG. 13. A later stage with a delicate chromatin network.
- FIG. 14. Formation of chromatin thread attached to the nucleolus.
- FIG. 15. A definite spireme.
- FIG. 16. Thread system entering synizesis.
- FIG. 17. Early synizesis showing material passing from the nucleolus into the threads.
- FIG. 18. Further condensation of chromatin material.

PLATE XXVIII

- FIG. 19. Mid-synizesis showing the contracted thread system.
- FIG. 20. Mid-synizesis.
- FIGS. 21 AND 22. Emerging from synizesis.
- FIG. 23. Open spireme.
- FIG. 24. Chromatin nodules on spireme.
- FIG. 25. Pairing of threads.
- FIG. 26. Paired threads twisted.
- FIG. 27. Second contraction, twisted threads.

- FIG. 28. Diakinesis, the chromosomes in pairs.
FIG. 29. Univalent chromosomes following diakinesis.

PLATE XXIX

- FIG. 30. Further condensation of chromosomes.
FIG. 31. Beginning of spindle formation.
FIG. 32. Homologous chromosomes becoming arranged in pairs on spindle.
FIG. 33. Homologous chromosomes closely paired.
FIG. 34. Polar view of equatorial plate showing pairing of univalents.
FIGS. 35 AND 36. Metaphase of the heterotypic division, the univalents closely paired.
FIG. 37. Polar view of later metaphase, the univalents beginning to separate.
FIG. 38. Anaphase of heterotypic mitosis.
FIG. 39. Late anaphase showing splitting of the chromosomes.
FIG. 40. The daughter chromosomes separating after the split in late anaphase.
FIGS. 41 AND 42. Interkinesis showing univalent chromosomes and remnants of thread connections.

PLATE XXX

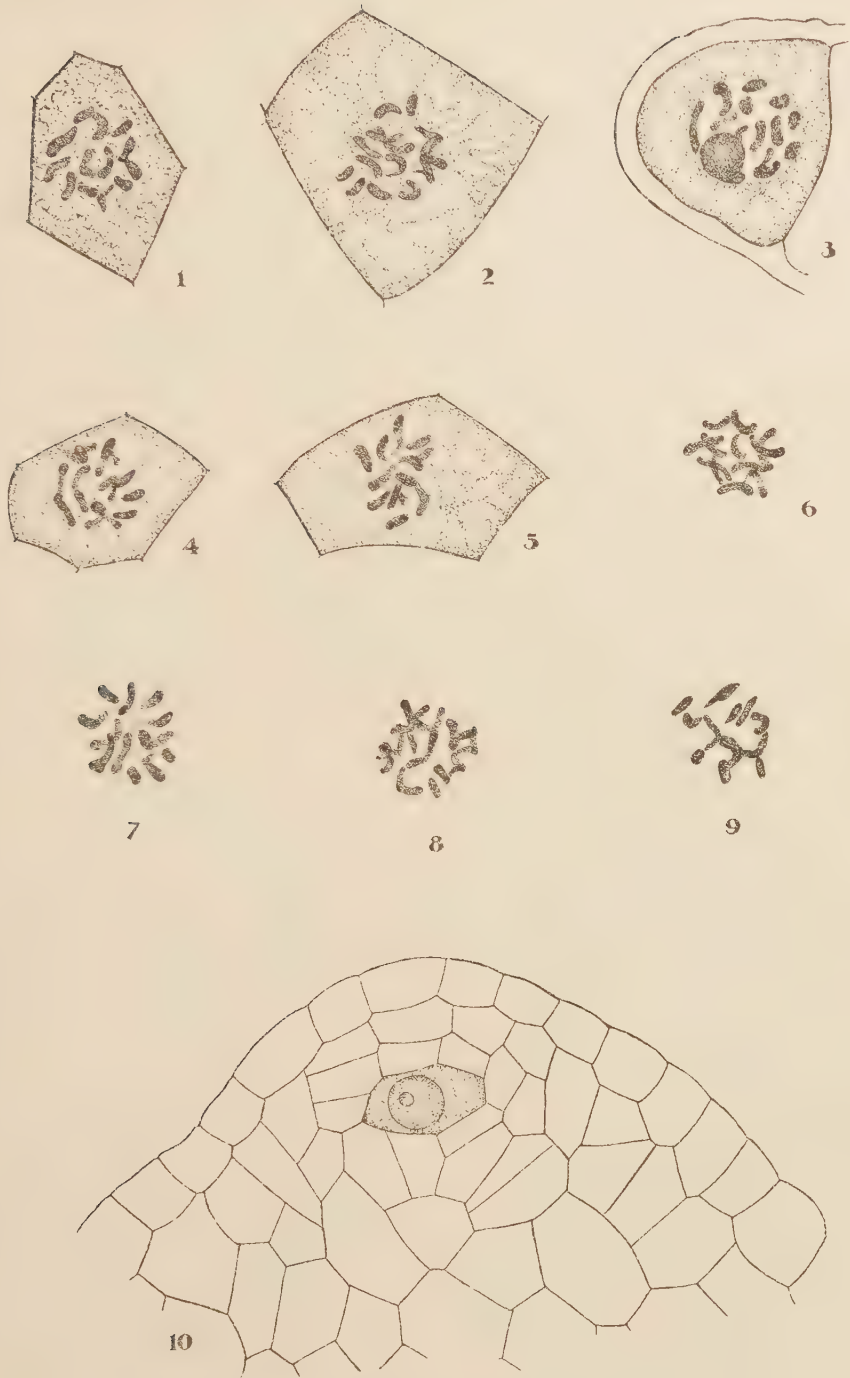
- FIG. 43. Daughter chromosomes becoming associated preliminary to the homoeotypic division.
FIG. 44. Homoeotypic metaphases.
FIG. 45. Homoeotypic anaphases.
FIG. 46. Nuclei of the tetrad with connecting fibers.
FIG. 47. Secondary wall formed inside the primary wall.
FIG. 48. Tetrad showing vacuolization at the equatorial planes.
FIG. 49. Secondary wall following the furrows that have cut out the microspores.
FIG. 50. Tetrad of microspores.

PLATE XXXI

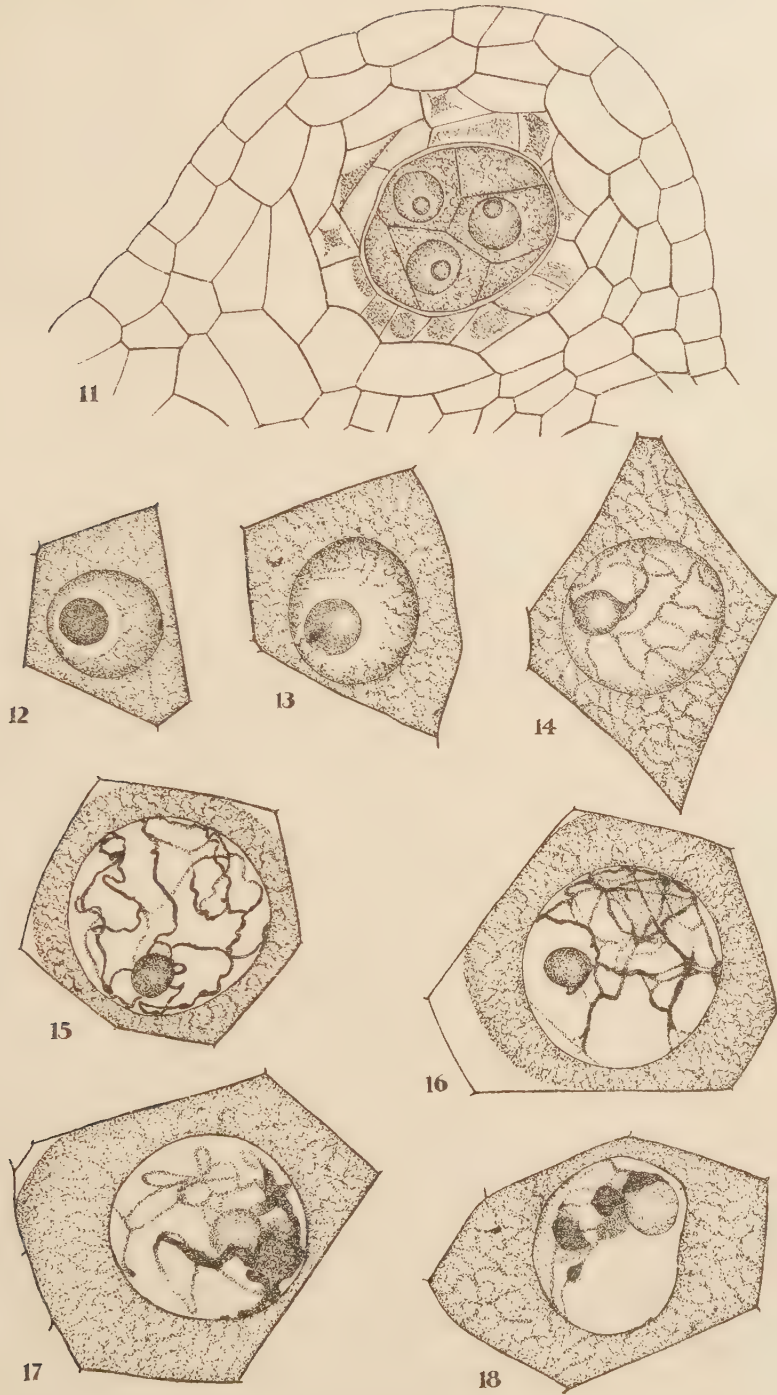
- FIGS. 51 AND 52. Synizesis in the megasporocyte.
FIG. 53. Spireme with chromatin nodules, following synizesis.
FIG. 54. Pairing of threads.
FIG. 55. Second contraction of chromatin material.
FIG. 56. Diakinesis, the chromosomes in pairs.
FIG. 57. Univalent chromosomes following diakinesis.
FIG. 58. Further condensation of chromosomes.
FIG. 59. Homologous chromosomes becoming arranged in pairs on the spindle.
FIGS. 60 AND 61. Metaphase of heterotypic division in the megasporocyte. s, odd chromosome.
FIG. 62. Interkinesis in the megasporocyte.

PLATE XXXII

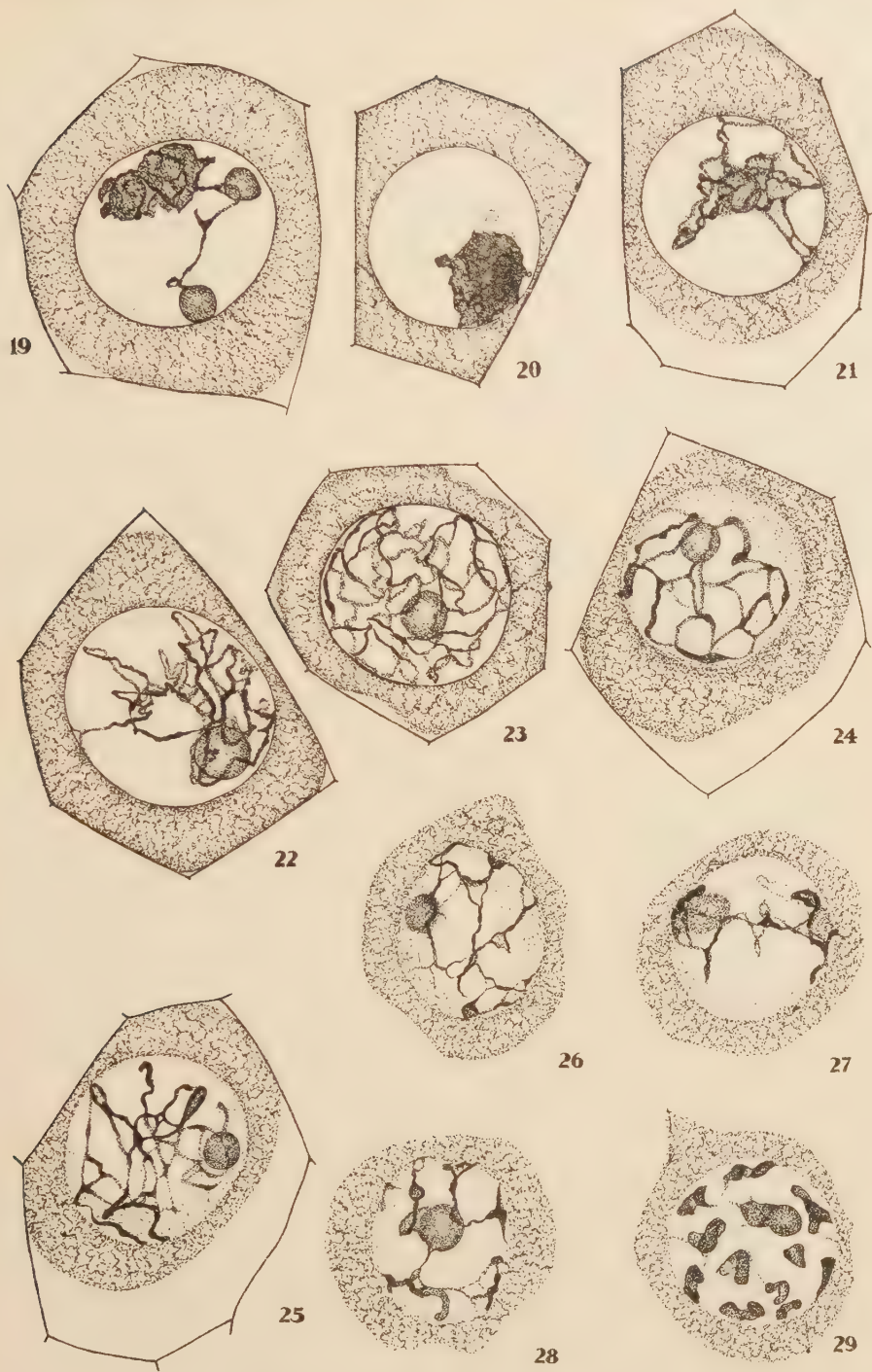
- FIG. 63. Metaphases of the homoeotypic division showing 6 chromosomes on one spindle and 7 chromosomes on the other.
FIG. 64. Metaphase of homeotypic division in one cell, anaphase in the other.
FIG. 65. Megaspores (tetrad) showing relation to two large cells of the nucellus at the micropylar end.



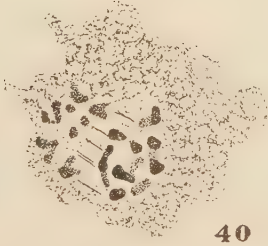
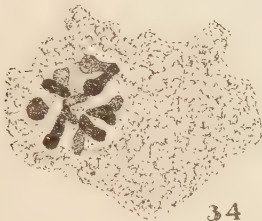
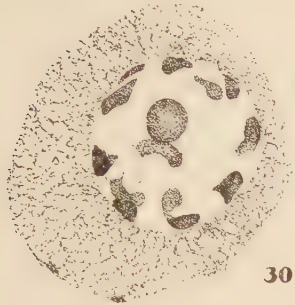
PASTRANA: SPOROGENESIS IN BEGONIA



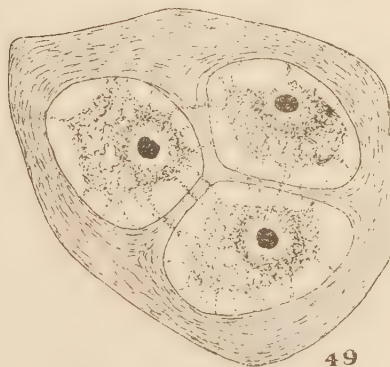
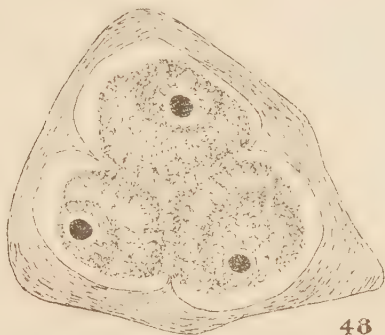
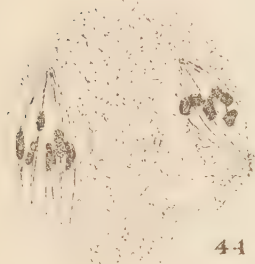
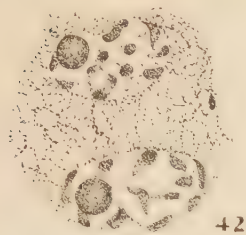
PASTRANA: SPOROGENESIS IN BEGONIA



PASTRANA: SPOROGENESIS IN BEGONIA

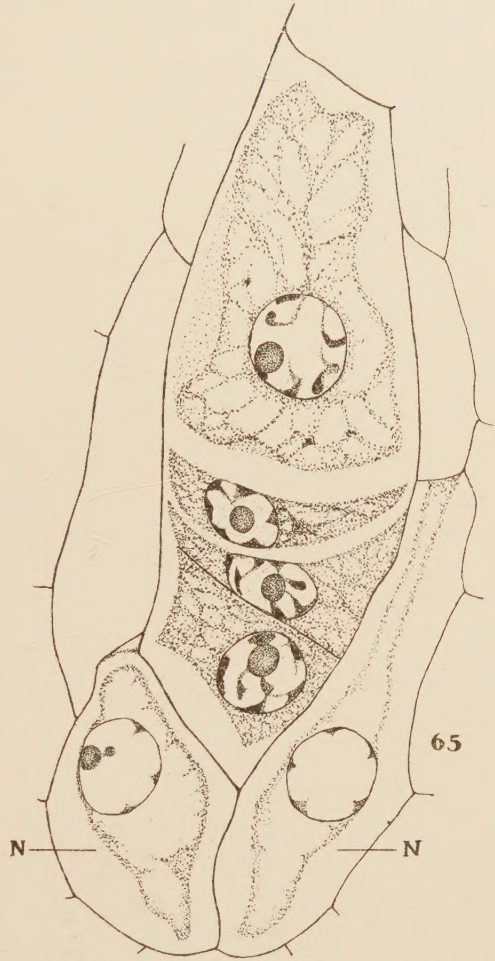
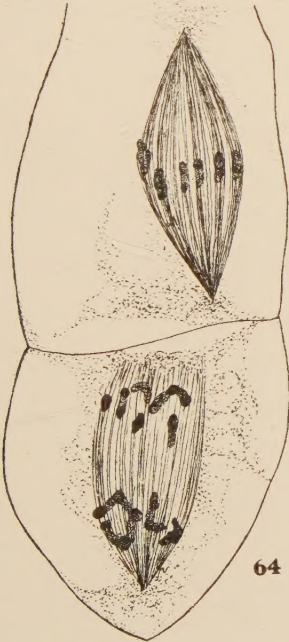
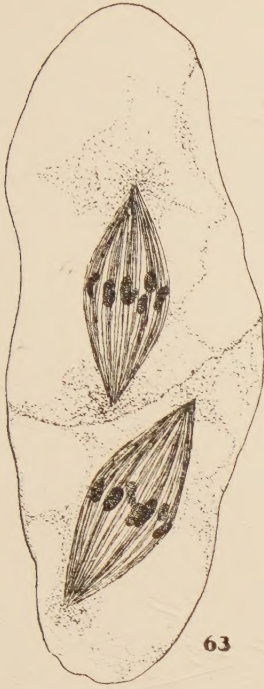


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